Cell Meter™ Mitochondrion Membrane Potential Assay Kit *Orange Fluorescence Optimized for Flow Cytometry*

Catalog number: 22804
Unit size: 100 Tests

Component	Storage	Amount
Component A: 500X MitoTell ™ Orange in DMSO	Freeze (<-15 °C), Minimize light exposure	1 vial (200 µL)
Component B: Assay Buffer	Freeze (<-15 °C)	1 bottle (100 mL)

OVERVIEW

This particular kit is designed to detect cell apoptosis by measuring the loss of the mitochondrial membrane potential(MMP). The collapse of mitochondrial membrane potential coincides with the opening of the mitochondrial permeability transition pores, leading to the release of cytochrome C into the cytosol, which in turn triggers other downstream events in the apoptotic cascade. Our Cell Meter™ Orange Mitochondrial Membrane Potential Assay Kit provides all the essential components with an optimized assay method. This fluorimetric assay uses our proprietary cationic MitoLite™ Orange for the detection of apoptosis in cells with the loss of mitochondrial membrane potential. In normal cells, the red fluorescence intensity is increased when MitoLite™ Orange is accumulated in the mitochondria. However, in apoptotic cells, the fluorescence intensity of MitoLite™ Orange is decreased following the collapse of MMP. Cells stained with MitoLite™ Orange can be visualized with a flow cytometer at 488 nm excitation with red emission (FL2 channel). The kit can be used together with other reagents, such as Cell Meter™ Phosphatidylserine Apoptosis Assay Kit (22835) for multi-parametric study of cell vitality and apoptosis. The kit is optimized for screening apoptosis activators and inhibitors with a flow cytometer.

AT A GLANCE

Protocol summary

1. Prepare cells with test compounds at the density of 5×10^5 to 1×10^6 cells/mL
2. Add 2 µL of 500X MitoTell™ Orange into 1 mL of cell solution
3. Incubate the cells in a 37 °C, 5% CO_2 incubator for 15 - 30 minutes
4. Pellet the cells, and resuspend the cells in 1 mL of growth medium
5. Analyze cells using flow cytometer with FL2 channel

Important Thaw all the kit components at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Flow cytometer
Excitation:	488 nm or 532 nm laser
Emission:	575/26 nm filter
Instrument specification(s):	PE channel

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit https://www.aatbio.com/resources/guides/cell-sample-preparation.html

SAMPLE EXPERIMENTAL PROTOCOL

1. For each sample, prepare cells in 1 mL of warm medium or buffer of your choice at the density of 5×10^5 to 1×10^6 cells/mL.

   Note Each cell line should be evaluated on an individual basis to determine the optimal cell density for apoptosis induction.

2. Treat cells with test compounds for a desired period of time to induce apoptosis, and set up parallel control experiments.

   For Negative Control: Treat cells with vehicle only.

   For Positive Control: Treat cells with FCCP or CCCP at 5-50 µM in a 37 oC, 5% CO_2 incubator for 15 to 30 minutes.

   Note CCCP or FCCP can be added simultaneously with MitoTell™ Orange. To get the best result, titration of the CCCP or FCCP may be required for each individual cell line.

3. Add 2 µL of 500X MitoTell™ Orange (Component A) into the treated cells.

4. Incubate the cells in a 37 °C, 5% CO_2 incubator for 15 to 30 minutes.

   Note For adherent cells, gently lift the cells with 0.5 mM EDTA to keep the cells intact and wash the cells once with serum-containing media prior to the incubation with MitoTell™ Orange dye-loading solution.

5. Centrifuge the cells at 1000 rpm for 4 minutes, and then re-suspend cells in 1 mL of Assay Buffer (Component B) or buffer of your choice.

6. Monitor the fluorescence intensity using a flow cytometer wih FL2 channel (Ex/Em = 540/590 nm). Gate on the cells of interest, excluding debris.

EXAMPLE DATA ANALYSIS AND FIGURES

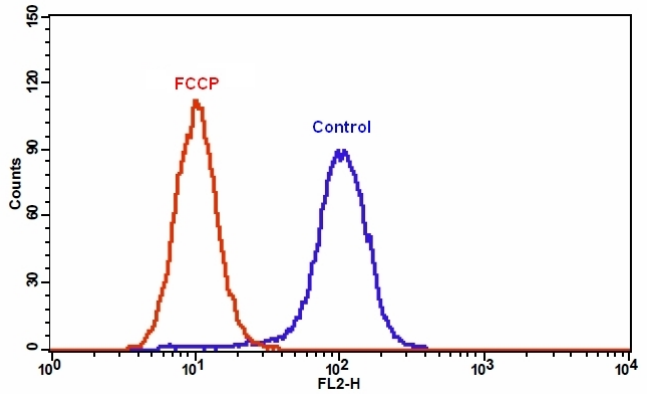

Figure 1. The decrease in fluorescence intensity of MitoTell™ Orange with the addition of FCCP in Jurkat cells. Jurkat cells were loaded with MitoTell™ Orange alone (Blue) or in the presence of 30 µM FCCP (Red) for 15 minutes. The fluorescence intensity of MitoTell™ Orange was measured with a FACSCalibur (Becton Dickinson, San Jose, CA) flow cytometer using FL2 channel.

DISCLAIMER